bio-protocol Preprint

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Colon loop ligation assay.

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Updated date: Jun 23, 2021

An abbreviated version of this protocol was published in Nat Microbiol in Jun 2019

Sulfated glycosaminoglycans and low-density lipoprotein receptor contribute to Clostridium difficile toxin A entry into cells

DOI: 10.1038/s41564-019-0464-z

Detailed protocol

1. Dissect out the colon from the adult mice after euthanizing the mice..
2. Wash the colon with cold PBS until the colon is clean completely.
3. Sealed ~2cm loop in the ascending colon with silk ligatures.
4. Inject 100ul Alexa555-labelled TcdA1–1874 or TcdA into the sealed colon segment using an intravenous catheter.
5. Incubate the sealed colon segments on ice for 30minutes.
6. Cut open both ends of the colon.
7. Wash the colon with cold PBS for 3 times
8. Use OCT to embed the colon
9. Section the colon tissue into10 μm thickness of sections.
10. Fix the sections with 4% PFA for 10 minutes
11. Wash the sections with PBS for 3 times
12. Mount the sections with fluoromount G with DAPI and secure with cover glass
13. Image the sections with Ultraview Vox Spinning Disk Confocal System (Olympus 71X).

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Tao, L. , Dong, M. and Zhang, J. (2021). Colon loop ligation assay.. Bio-protocol Preprint. bio-protocol.org/prep1196.
2. Tao, L., Tian, S., Zhang, J., Liu, Z., Robinson-McCarthy, L., Miyashita, S., Breault, D. T., Gerhard, R., Oottamasathien, S., Whelan, S. and Dong, M.(2019). Sulfated glycosaminoglycans and low-density lipoprotein receptor contribute to Clostridium difficile toxin A entry into cells. Nat Microbiol 4(10). DOI: 10.1038/s41564-019-0464-z

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